PATENT COOPERATION TREATY

PCT

INTERNATIONAL PRELIMINARY REPORT ON PATENTABILITY

(Chapter II of the Patent Cooperation Treaty)

(PCT Article 36 and Rule 70)

Applicant's or agent's file reference 33686 PC 01	**FOR FURTHER ACTION**	See Form PCT/IPEA/416
International application No. PCT/DK2005/000068	International filing date *(day/month/year)* 30.01.2005	Priority date *(day/month/year)* 30.01.2004
International Patent Classification (IPC) or national classification and IPC INV. C12N9/16 A61K38/46		
Applicant ZYMENEX A/S et al.		

1. This report is the international preliminary examination report, established by this International Preliminary Examining Authority under Article 35 and transmitted to the applicant according to Article 36.
2. This REPORT consists of a total of 9 sheets, including this cover sheet.
3. This report is also accompanied by ANNEXES, comprising:
   a. ☒ *sent to the applicant and to the International Bureau)* a total of 3 sheets, as follows:
      ☒ sheets of the description, claims and/or drawings which have been amended and are the basis of this report and/or sheets containing rectifications authorized by this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions).
      ☐ sheets which supersede earlier sheets, but which this Authority considers contain an amendment that goes beyond the disclosure in the international application as filed, as indicated in item 4 of Box No. I and the Supplemental Box.
   b. ☐ *(sent to the International Bureau only)* a total of (indicate type and number of electronic carrier(s)) , containing a sequence listing and/or tables related thereto, in electronic form only, as indicated in the Supplemental Box Relating to Sequence Listing (see Section 802 of the Administrative Instructions).

4. This report contains indications relating to the following items:

☒	Box No. I	Basis of the report
☒	Box No. II	Priority
☐	Box No. III	Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
☐	Box No. IV	Lack of unity of invention
☒	Box No. V	Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
☐	Box No. VI	Certain documents cited
☐	Box No. VII	Certain defects in the international application
☒	Box No. VIII	Certain observations on the international application

Date of submission of the demand	Date of completion of this report
30.11.2005	18.05.2006
Name and mailing address of the international preliminary examining authority: European Patent Office D-80298 Munich Tel. +49 89 2399 - 0 Tx: 523656 epmu d Fax: +49 89 2399 - 4465	Authorized officer Morawetz, R Telephone No. +49 89 2399-8155

INTERNATIONAL PRELIMINARY REPORT ON PATENTABILITY

International application No.
PCT/DK2005/000068

Box No. I Basis of the report

1. With regard to the **language**, this report is based on

   ☒ the international application in the language in which it was filed

   ☐ a translation of the international application into , which is the language of a translation furnished for the purposes of:
   - ☐ international search (under Rules 12.3(a) and 23.1(b))
   - ☐ publication of the international application (under Rule 12.4(a))
   - ☐ international preliminary examination (under Rules 55.2(a) and/or 55.3(a))

2. With regard to the **elements*** of the international application, this report is based on *(replacement sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to this report)*:

   Description, Pages

   1-85 as originally filed

   Sequence listings part of the description, Pages

   1-5 as originally filed

   Claims, Numbers

   1-17 filed with telefax on 03.05.2006

   Drawings, Sheets

   1/17-17/17 as originally filed

   ☒ a sequence listing and/or any related table(s) - see Supplemental Box Relating to Sequence Listing

3. ☐ The amendments have resulted in the cancellation of:
   - ☐ the description, pages
   - ☐ the claims, Nos.
   - ☐ the drawings, sheets/figs
   - ☐ the sequence listing *(specify)*:
   - ☐ any table(s) related to sequence listing *(specify)*:

4. ☐ This report has been established as if (some of) the amendments annexed to this report and listed below had not been made, since they have been considered to go beyond the disclosure as filed, as indicated in the Supplemental Box (Rule 70.2(c)).
   - ☐ the description, pages
   - ☐ the claims, Nos.
   - ☐ the drawings, sheets/figs
   - ☐ the sequence listing *(specify)*:
   - ☐ any table(s) related to sequence listing *(specify)*:

* *If item 4 applies, some or all of these sheets may be marked "superseded."*

INTERNATIONAL PRELIMINARY REPORT ON PATENTABILITY

International application No.
PCT/DK2005/000068

Box No. II Priority

1. ☐ This report has been established as if no priority had been claimed due to the failure to furnish within the prescribed time limit the requested:
   ☐ copy of the earlier application whose priority has been claimed (Rule 66.7(a)).
   ☐ translation of the earlier application whose priority has been claimed (Rule 66.7(b)).

2. ☐ This report has been established as if no priority had been claimed due to the fact that the priority claim has been found invalid (Rule 64.1). Thus for the purposes of this report, the international filing date indicated above is considered to be the relevant date.

3. Additional observations, if necessary:

   see separate sheet

Box No. V Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Yes:	Claims	1-12, 14, 15
	No:	Claims	13, 16, 17
Inventive step (IS)	Yes:	Claims	1-12
	No:	Claims	14, 15
Industrial applicability (IA)	Yes:	Claims	1-17
	No:	Claims	

2. Citations and explanations (Rule 70.7):

   see separate sheet

Box No. VIII Certain observations on the international application

The following observations on the clarity of the claims, description, and drawings or on the question whether the claims are fully supported by the description, are made:

see separate sheet

Supplemental Box relating to Sequence Listing

Continuation of Box I, item 2:

1. With regard to any nucleotide and/or amino acid sequence disclosed in the international application and necessary to the claimed invention, this report was established on the basis of:

   a. type of material:

   ☒ a sequence listing

   ☐ table(s) related to the sequence listing

   b. format of material:

   ☒ on paper

   ☒ in electronic form

   c. time of filing/furnishing:

   ☒ contained in the international application as filed

   ☒ filed together with the international application in electronic form

   ☐ furnished subsequently to this Authority for the purposes of search and/or examination

   ☐ received by this Authority as an amendment* on

2. ☐ In addition, in the case that more than one version or copy of a sequence listing and/or table(s) relating thereto has been filed or furnished, the required statements that the information in the subsequent or additional copies is identical to that in the application as filed or does not go beyond the application as filed, as appropriate, were furnished.

3. Additional comments:

* *If item 4 in Box No. I applies, the listing and/or table(s) related thereto, which form part of the basis of the report, may be marked "superseded."*

Re Item I

Basis of the report

1.1. The amendments filed with the fax dated 03.05.2006 appear allowable under Article 34(2)(b) PCT.

1.2. The applicant is however requested to note that claim 1, lines 12-13 should read "anion exchange membrane" and not "anion chromatography membrane", see original claim 17. Claim 16 , line 1 should read "medicament" and not "formulation", see original claim 31.

Re Item II

Priority

Present application is not entirely entitled to the claimed priority.

Re Item V

Reasoned statement with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Cited documents

Reference is made to the documents cited in the international search report.

D1: WO 02/098455 A (FOGH JENS ; HEMEBIOTECH AS (DK); ANDERSSON CLAES (SE); WEIGELT CECILIA) 12 December 2002 (2002-12-12)

D2: WO 02/40686 A (GENZYME CORP) 23 May 2002 (2002-05-23)

D3: SARAFIAN T A ET AL., BIOCHEMICAL MEDICINE, ACADEMIC PRESS, SAN DIEGO, CA, US, vol. 33, no. 3, 1985, pages 372-380

D4: STEVENS R L ET AL., JOURNAL OF BIOLOGICAL CHEMISTRY, vol. 250, no. 7, 1975, pages 2495-2501, XP002300885 ISSN: 0021-9258

D5: BOSTICK W D ET AL., CLINICAL CHEMISTRY, AMERICAN ASSOCIATION FOR CLINICAL CHEMISTRY. WINSTON, US, vol. 24, no. 8, 1978, pages 1305-1316, XP009036852 ISSN: 0009-9147

D6: STEIN C ET AL., JOURNAL OF BIOLOGICAL CHEMISTRY. (MICROFILMS), AMERICAN SOCIETY OF BIOLOGICAL CHEMISTS, BALTIMORE, MD, US, vol. 264, no. 2, 15 January 1989 (1989-01-15), pages 1252-1259, XP002185735

D7: SANGALLI A ET AL., HUMAN GENE THERAPY, XX, XX, vol. 9, no. 14, 20 September 1998 (1998-09-20), pages 2111-2119

D8: MATZNER U ET AL., GENE THERAPY, vol. 9, no. 1, January 2002 (2002-01), pages 53-63, XP002322286 ISSN: 0969-7128

D9: KAKKIS E ET AL., JOURNAL OF INHERITED METABOLIC DISEASE, KLUWER, DORDRECHT, NL, vol. 26, no. SUPPL 2, September 2003 (2003-09), page 141, XP009036788 ISSN: 0141-8955

2. Subject-matter of the application

Present application relates to a process for production and purification of recombinant arylsulfatase A (rASA) in a continuous cell culture system and the use of the rASA for preventing or alleviating the symptoms related to Metachromatic leukodystrophy (MLD). MLD is caused by an autosomal recessive genetic defect in the lysosomal enzyme Arylsulfatase A (ASA).

3. Novelty

3.1. The present application does not meet the criteria of Article 33(1) PCT, because the subject-matter of claims 13, 16 and 17 is not new in the sense of Article 33(2) PCT.

3.2. The document **D1** discloses (the references in parentheses applying to this document) a process for production and purification of recombinant human arylsulfatase A (rhASA). It also discloses (page 9, lines 16-21) a method for preventing or treating the development of symptoms related to MLD by administering an effective amount of ASA or an enzymatically equivalent part or analogue of it. Delivery across the blood-brain-barrier (BBB) and to oligodendrocytes in the brain is likewise disclosed (page 9, lines 23-35). Delivery over a cellular membrane, to a target cell is achieved by taking advantage of a mannose-receptor-mediated uptake. Thus mannose-6-phosphate tagged ASA is made in a mammalian cells system (e.g.

CHO, COS cells or BHK cells) to secure correct mannose-6-phosphate tagging on the molecule and the mannose-6-phosphate tagged ASA is secreted into the medium (page 12, lines 11-18). The rhASA of D has an activity of 20-25U/mg (page 41, lines 11-17) or 30-50 U/mg (page 42, lines 6-13). D1 also discloses (page 9, line 23 - page 10, line 7) a treatment method in which a cellular barrier such as the blood-brain-barrier is crossed whereby the material is delivered to the target cells. Preferably, a vehicle such as a modified form of the protein, a peptide, or fragments thereof and/or modified functional domains of toxins or fragments thereof will carry the material to the target cells. It is contemplated that effective enzyme replacement therapy of MLD patients with recombinant human ASA (rhASA) will require uptake of an active enzyme into the target cells such as the myelin forming cells (oligodendrocytes) of the brain. To be able to deliver rhASA to the brain a vehicle that can pass the blood-brain-barrier (BBB) is likely to be needed since rhASA is not likely to be able to traverse over the BBB by it self. D1 however also discloses (page 10, lines 4-5) that enzymes can be delivered to oligodendrocytes in the brain directly via the cerebral spinal fluid (CSF).

D1 thus anticipates the subject-matter of claims 13, 16 and 17.

3.3. The subject-matter of claims 1-12, 14 and 15 appears to be novel.

4. Inventive step

4.1. The present application does not meet the criteria of Article 33(1) PCT, because the subject-matter of claims 14 and 15 does not involve an inventive step in the sense of Article 33(3) PCT.

4.2. Dependent claims 14 and 15 do not contain any technical features which in combination with the features of claim 13 meet the requirements of Article 33(3) PCT.

4.2. The subject-matter of claims 1-12 does involve an inventive step in the sense of Article 33(3) PCT.

Claim 1 relates to a process for production of rASA in a continuous cell culture

system, the process comprising: (i) culturing a mammalian cell capable of producing arylsulfatase A in liquid medium in a system comprising one or more bio-reactors; (ii) concentrating, purifying and formulating the rhASA by a purification process comprising one or more steps of affinity chromatography and/or ion exchange chromatography; wherein the concentration and purification process of (ii) comprises a polishing step including a passive step, wherein the arylsulfatase A passes through a cation chromatography resin or membrane, and an active step, wherein the arylsulfatase A is detained within and subsequently eluted from an anion exchange membrane or resin, and wherein the cation chromatography resin or membrane and the anion exchange membrane or resin are coupled or connected in a series.

The document **D1**, which is regarded as being the closest prior art to the subject-matter of claim 1, discloses (the references in parentheses applying to this document) a process for the production of rASA in a semi-large scale fermentation comprising culturing a CHO-ASA cell line in a 5 litre bioreactor followed by a purification process comprising several steps of ion exchange chromatography and a polishing step (Examples 5 and 6).

The subject-matter of claim 1 therefore differs from this known process in that the production occurs in a continuous process and comprises a polishing step including a passive and an active step on cation chromatography resin and an anion exchange resin, respectively, and wherein the cation chromatography resin or membrane and the anion exchange membrane or resin are coupled or connected in a series.

The problem to be solved by the present invention may therefore be regarded as the provision of an alternative process for the production of recombinant arylsulphatase A.

The solution proposed in claim 1 of the present application is considered as being novel and as involving an inventive step (Article 33(3) PCT) since it is neither disclosed nor suggested by the prior art.

Re Item VIII

Certain observations on the international application

The application does not meet the requirements of Articles 5 and 6 PCT, because the subject-matter of claim 1 and others is neither sufficiently clear and complete disclosed in the description nor supported by the description. It is quite clear from the description (page 24, line 21 - page 10, line 10) that the polishing step takes advantage of unexpected characteristics of human rASA, namely its property to bind to cation exchangers but also to positively charged anion exchangers at pH 4.8. The application does not disclose that any other rASA will behave similarly, to the contrary it is stated that it is expected that very few other proteins will behave similarly. According to the description the polishing step is initiated at pH 6.0 where the enzyme will not bind to a first affinity chromatography resin or a first cation exchanger. Elution from the anion exchanger takes place at pH around 4.8. These features are essential features of the invention and have thus to be present in independent claim 1 for it to fulfill the requirements of Articles 5 and 6 PCT.

33686pc01

CLAIMS

1. A process for production of recombinant arylsulphatase A in a continuous cell culture system, the process comprising:

i) culturing a mammalian cell capable of producing arylsulfatase A in liquid medium in a system comprising one or more bio-reactors;

ii) concentrating, purifying and formulating the rhASA by a purification process comprising one or more steps of affinity chromatography and/or ion exchange chromatography;

wherein the concentration and purification process of (ii) comprises a polishing step including a passive step, wherein the arylsulfatase A passes through a cation chromatography resin or membrane, and an active step, wherein the arylsulfatase A is detained within and subsequently eluted from an anion exchange membrane or resin, and wherein the cation chromatography resin or membrane and the anion chromatography membrane or resin are coupled or connected in a series.

2. A process according to claim 1, wherein said mammalian cell comprises a nucleic acid sequence, which encodes:

(a) the amino acid sequence of SEQ ID NO:2;

(b) a portion of the sequence in (a), which is enzymatically equivalent to recombinant human arylsulfatase A

(c) an amino acid sequence analogue having at least 75% sequence identity to any one of the sequences in (a) or (b) and at the same time comprising an amino acid sequence, which is enzymatically equivalent to recombinant human arylsulfatase A.

3. A process according to any of the preceding claims, wherein the arylsulfatase A produced is selected from the group consisting of

(a) the amino acid sequence of SEQ ID NO:3;

(b) a portion of the sequence in (a), which is enzymatically equivalent to recombinant human arylsulfatase A

(c) an amino acid sequence analogue having at least 75% sequence identity to any one of the sequences in (a) or (b) and at the same time being enzymatically equivalent to recombinant human arylsulfatase A.

4. A process according to any of the preceding claims, wherein the mammalian cells are of human or primate origin.

5. A process according to any of the preceding claims, wherein the concentration and purification process of ii) comprises one or more steps of Expanded Bed Chromatography.

6. A process according to any of the preceding claims, wherein the concentration and purification process of ii) comprises the following steps:

II) contacting an arylsulfatase A containing supernatant on an equilibrated chromatography column and eluting one or more fraction(s) containing arylsulfatase A;

III) loading the fraction(s) from step II on another equilibrated chromatography column and eluting one or more fraction(s) containing arylsulfatase A;

IV) buffer exchange of the arylsulfatase A present in the fraction(s) from step III by tangential flow filtration;

V) polishing the preparation of arylsulfatase A from step IV in one or two or more successive steps, each step comprising loading the preparation on an equilibrated chromatography columns and eluting one or more fraction(s) containing arylsulfatase A;

VI) passing the fraction(s) from step V through a viral reduction filter;

VII) formulating the fraction(s) from step VI in order to obtain a preparation of arylsulfatase A in a suitable formulation buffer;

VIII) optionally filling the formulated preparation of arylsulfatase A into a suitable container and freeze-drying the sample.

7. A process according to claim 6, further comprising an initial step I) of concentrating the arylsulfatase A by tangential flow filtration.

8. A process according to any of claims 6 or 7, wherein the chromatography column used in step II of the purification process is an anion exchange column.

9. A process according to claim 8, wherein said anion exchange column is a DEAE Sepharose column or a DEAE Streamline column.

10. A process according to any of claims 6 to 9, wherein the chromatography column used in step III of the purification process is a hydrophobic interaction column.

11. A process according to any of claims 6 to 10, wherein purification of the sample in step IV of the purification process is accomplished by tangential flow filtration.

12. A process according to any of claims 6 to 12, wherein the filtration of the sample as performed in step VI of the purification process is replaced by or combined with contacting

the sample with a detergent, preferably prior to step V or preferably prior to step II of the purification process.

13. Use of a formulation comprising an effective amount of arylsulfatase A for the manufacture of a medicament for reducing the levels of galactosyl sulphatide in cells within the central nervous system in a subject suffering from and/or being diagnosed with metachromatic leukodystrophy, wherein said formulation is obtainable by a process according to any of claims 1-12, and wherein said formulation is to be administered by a route other than intracerebroventricular, spinal, intrathecal or intracranial administration

14. Use according to claim 13, wherein said formulation is to be administered by intravenous or subcutaneous administration.

15. Use according to claim 13 or 14, wherein said formulation is to be administered on a daily, weekly, bi-weekly or monthly basis.

16. Use according to any of claims 13-15, wherein said formulation is for administration to a subject which do not receive any additional medical treatment for reduction of the sphingolipid 3-O-sulfogalgactosylceramide levels, including:

a) administration a formulation comprising a vehicle, such as a peptide or polypeptide, for delivery of the enzyme (arylsulfatase A) into the central nervous system, and

b) administration of a formulation capable of causing opening or disruption of the blood brain barrier, and

c) administration of an intact cell.

17. Use according to any of claims 13-16, wherein said formulation does not comprise any of the following:

a) a vehicle, such as a peptide or polypeptide, for delivery of aryl sulfatase A into the central nervous system, and

b) a component capable of causing opening or disruption of the blood brain barrier, and

c) an intact cell